

Scrapie infection can be established readily through skin scarification in immunocompetent but not immunodeficient mice

D. M. Taylor, I. McConnell and H. Fraser

BBSRC & MRC Neuropathogenesis Unit, Institute for Animal Health, West Mains Road, Edinburgh EH9 3JF, UK

Scarification of the skin is a possible route of entry for scrapie infectivity in sheep, and for Creutzfeldt–Jakob disease agent in humans within the context of occupational exposure to infected brain in the autopsy room or laboratory. The effectiveness of skin scarification routes as portals of entry for infectivity had not previously been tested experimentally but this study has shown that these are efficient routes for establishing infection in mice using the 139A and ME7 strains of scrapie agent. Scarification had much the same efficiency as inoculation by the intraperitoneal, intravenous or perivenous routes but was not effective in immunocompromised (SCID) mice. It was concluded that replication of infectivity within the lymphoreticular system, which is precluded in SCID mice, is a necessary prerequisite for the development of infection in the central nervous system following inoculation via scarification.

The fatal sheep disease scrapie is a transmissible degenerative encephalopathy resulting from infection by the same type of unconventional agent that causes Creutzfeldt–Jakob disease (CJD) and kuru in humans, bovine spongiform encephalopathy (BSE) and transmissible mink encephalopathy (TME). Scrapie is perpetuated principally by maternal transmission, possibly as a result of perinatal contact with the infected placenta, but it has been noted that 'the role of the placenta in either vertical or lateral transmission of scrapie in sheep is a matter for speculation' (Dickinson & Fraser, 1979). Specific routes whereby perinatal infection is initiated have not been established but experiments have shown that administering infectivity to sheep through the conjunctiva (Haralambiev *et al.*, 1973) or by oral dosing (Pattison & Millson,

1961; Gordon, 1966*a, b*; Pattison *et al.*, 1974) can produce disease. Further possible routes of entry might be through the unhealed umbilical stump, or skin which became damaged around the time of birth. The potential for infection to be established through skin scarification has not previously been tested experimentally.

This study was designed to determine the effectiveness of establishing experimental scrapie infection in mice by inoculating infectivity through skin scarification compared with other routes of inoculation. The scarification route may also have relevance to the less common situation where scrapie appears to be transmitted in adulthood either as a result of contact in the field with known cases of scrapie (Brotherston *et al.*, 1968; Haralambiev *et al.*, 1973; Dickinson *et al.*, 1974) or by exposure to pastures contaminated putatively with scrapie agent (Greig, 1950; Palsson, 1979). In neither of these scenarios is the route of entry of infectivity into the host known. There is also the question as to whether CJD infection might be acquired occupationally through scarification while handling infected human brain in the autopsy room or laboratory. CJD has occurred in healthcare workers but without any clear association with high risk of exposure, even though infected brains were handled without precautions for many years (Taylor, 1993). Nevertheless, the possibility of establishing CJD infection through trauma has been demonstrated by the occurrence of the disease in patients who received intramuscular injections of CJD-contaminated human growth hormone (Brown *et al.*, 1992). There is also considered to be a possible role for skin trauma in establishing TME infection. The disease has a putative foodborne source but it is considered that the infectivity may gain access to mink most successfully through the biting which occurs especially during feeding (Marsh & Hanson, 1979). Kuru has been confined to the Fore tribal population of New Guinea, and acquisition of the disease was particularly associated with the ritualistic practice of removing and handling brains of deceased relatives. There are reports of cannibalism but these are uncorroborated (Taylor, 1989), and Gajdusek (1985) states that the infection was 'most probably through cuts and abrasions of the skin or from nose-picking, eye-rubbing or mucosal injury'.

The inocula for the experiments in this study were 1% or

Author for correspondence: D. M. Taylor.

Fax +44 131 668 3872. e-mail David-M.Taylor@BBSRC.ac.uk

Table 1. Titration of ME7 in mice by different inoculation routes

Mouse strain	Route and dose	Dilution of inoculum	No. affected/ no. inoculated	Mean incubation period in days (se)	Titre (ID ₅₀ /g)
SM	i.c. (20 µl)	10 ⁻³	8/8	159 (2)	≥ 10 ^{8.1}
		10 ⁻⁴	7/7	179 (2)	
		10 ⁻⁵	12/12	201 (4)	
		10 ⁻⁶	8/12	210 (2)	
		10 ⁻⁷	2/11	257 (9)	
SM	i.p. (20 µl)	10 ⁻¹	5/5	238 (3)	≥ 10 ^{6.8}
		10 ⁻²	6/6	265 (11)	
		10 ⁻³	6/6	280 (7)	
		10 ⁻⁴	12/12	304 (6)	
		10 ⁻⁵	6/10	294 (18)	
SM	i.v. (20 µl)	10 ⁻¹	4/4	195 (5)	≥ 10 ^{6.7}
		10 ⁻²	5/5	240 (9)	
		10 ⁻³	4/6	265 (5)	
		10 ⁻⁴	10/10	305 (7)	
		10 ⁻⁵	9/11	329 (10)	
SM	Scarification of medial right thigh (6 µl)	10 ⁻¹	6/6	297 (6)	≥ 10 ^{6.3}
		10 ⁻²	11/11	303 (7)	
		10 ⁻³	10/10	317 (6)	
		10 ⁻⁴	6/12	328 (8)	
		10 ⁻⁵	1/12	316	
C57	Scarification of medial right thigh (6 µl)	10 ⁻²	6/6	323 (7)	10 ^{6.7}
		10 ⁻³	5/5	331 (21)	
		10 ⁻⁴	5/5	341 (8)	
		10 ⁻⁵	0/3		

10% saline homogenates of mouse brain infected with the 139A or ME7 strains of scrapie agent. Where titration of infectivity in the inoculum was carried out, further serial tenfold dilutions were inoculated. Mice were inoculated by the intracerebral (i.c.), intraperitoneal (i.p.), intravenous (i.v.) or perivenous (p.v.) routes, or by scarification. The dorsal tail vein was the site for i.v. inoculation, and the location for p.v. inoculation was adjacent to this vessel. The site used for scarification was usually the medial surface of the right thigh but in some experiments scarification was done alternatively at the axial surface of the right thigh, the medial surface of the left thigh, the dorsal lumbar area or the scruff of the neck. Mice were anaesthetized before scarification. The hair covering approximately 1 cm² of the area to be scarified was trimmed with scissors, and then removed completely with an electric shaver. The standard procedure thereafter was to deliver one droplet (6 µl) of scrapie-infected inoculum from a syringe with a 26 gauge needle onto the middle of the shaved area. Working through the droplet, the skin was abraded with sweeping strokes using a 26 gauge syringe-needle until minute droplets of blood could be observed, but without overt bleeding. Residual moisture was evaporated using a hair-drier. The scarification site was then sealed with Nobecutane surgical wound sealant (AB Astra), and the animals were not placed in

their final holding cages until this had dried. In one experiment, scarification was done before the inoculum was applied. In another, the skin was depilated, and inoculum and Nobecutane were applied but without scarification. The standard mouse strains used were BSC, C57BL and SM. Additionally, immunodeficient SCID (BALB/c, CB17 *scid/scid*) mice were used in one experiment together with immunocompetent CB20 +/+ congenic BALB/c mice. Mice were inoculated as weanlings, and scored for the development of clinical signs of scrapie by an established protocol (Dickinson *et al.*, 1968), at which point the affected animals were culled. Those animals which failed to develop scrapie were culled at ≥ 460 days after inoculation. The brains from all animals were processed histologically; coronal sections representing five areas were stained with haematoxylin and eosin and examined microscopically for spongiform encephalopathy (Fraser & Dickinson, 1968). Where titration of infectivity was carried out, the calculated titre was based upon the ratio of affected to unaffected mice according to the method of Karber (1931). Uninoculated mice were also placed in many of the cages housing the scarified animals to establish whether within-cage transmission could occur through adventitious contamination by the escape of scrapie infectivity from the scarification sites.

Table 1 shows that the i.c., i.p., i.v. and scarification routes

Table 2. Transmission of 139A to mice by different routes

Mouse strain	Route and dose*	No. affected/ no. inoculated	Mean incubation period in days (SE)
BSC	i.v. (20 µl)	9/9	173 (3)
	i.p. (20 µl)	6/6	187 (0)
	Scarification (6 µl)	13/18	191 (3)
	p.v. (20 µl)	12/12	193 (3)
SM	i.v. (20 µl)	6/6	206 (3)
	i.p. (20 µl)	6/6	213 (4)
	Scarification (6 µl)	10/10	218 (4)
	p.v. (20 µl)	5/5	225 (4)

* The original 10^{-2} inoculum was diluted so that each dose contained the same amount of infectivity regardless of dose volume.

were all successful in establishing infection with the ME7 strain of scrapie agent in SM mice. Scarification was also effective in C57 mice. Lower titres were observed following non-neural compared with i.c. inoculation, and this accords with existing data (Kimberlin & Walker, 1983). The incubation periods for the scarified mice were longer than for any of the other routes of inoculation but the volume of the inoculum was threefold less than for the other groups, and it was evident that not all of the inoculum could be taken up by the scarified mice. Nevertheless, the number of affected animals in the scarified group was comparable to those in the other non-neural inoculation groups; correspondingly, the titre of infectivity in all non-neural inoculation groups was comparable.

Table 2 demonstrates that the i.p., i.v., p.v. and scarification routes were all effective in establishing infection with the 139A

strain of scrapie agent in BSC and SM mice. In contrast to the experiments summarized in Table 1, the inocula for the non-scarification routes shown in Table 2 were diluted so that each injection dose (20 µl) had the same amount of infectivity as that contained in each 6 µl dose administered by scarification. As a result, the incubation periods for the scarified groups were much closer to those for the i.p., i.v. and p.v. groups compared with the data in Table 1. Not all of the scarified BSC mice developed scrapie but this was likely to represent technical failure rather than inefficiency of the scarification route in such mice because the mean incubation period for the affected animals was closer to those for the other peripheral inoculation routes in BSC mice (as it also was in the SM mice where all of the scarified animals developed scrapie).

The scarification site used in the experiments in Tables 1 and 2 was the medial surface of the right thigh. Table 3 shows that other scarification sites were equally effective; these were the axial surface of the right thigh, the medial surface of the left thigh, the dorsal lumbar area and the scruff of the neck. It was also demonstrated that placing inoculum on already scarified skin was just as effective in establishing infection as scarifying once the inoculum had been applied to the skin. Inoculum applied to shaved but unscarified skin did not cause infection. In the course of the experiments which are summarized in Table 3, 16 uninoculated mice were placed in the cages containing scarified mice. None developed scrapie, indicating that there had been no contagious spread attributable to leakage of infectivity from scarification sites.

These experiments have shown that infection can be established readily when scrapie agent is inoculated by scarification but it was not possible to conclude how infectivity reached the central nervous system (CNS) although transport via the bloodstream or transport via peripheral nerves are obvious possibilities. Scrapie is a neurodegenerative disease resulting from the replication of the infectious agent in the

Table 3. Transmission of ME7 to mice by scarification at different sites using 6 µl doses of 1% infected brain homogenate

Mouse strain	Scarification site*	Scarification method	No. affected/ no. inoculated	Mean incubation period in days (SE)
C57	mrt	Standard	5/5	313 (12)
SM	mrt	Standard	6/6	323 (7)
SM	art	Standard	5/5	295 (9)
SM	mlt	Standard	5/5	297 (3)
SM	dl	Standard	5/5	293 (5)
SM	n	Standard	7/7	321 (13)
SM	mrt	Scarification first	2/2	313 (14)
SM	mrt	No scarification	0/2	

* The sites were the medial surface of the right thigh (mrt), the axial surface of the right thigh (art), the medial surface of the left thigh (mlt), the dorsal lumbar area (dl) or the scruff of the neck (n)

CNS but infectivity is first detectable in other systemic tissues (particularly those of the lymphoreticular system) in which high titres are achieved but without any obvious clinical, cellular or immunological dysfunction. This is true not only for the natural disease in sheep (Hadlow *et al.*, 1979) but also for experimental scrapie in mice regardless of the route (including i.c.) of inoculation (Kimberlin & Walker, 1983). Following i.p. inoculation of a moderate dose of scrapie agent into mice, there appears to be the need for a stage of peripheral pathogenesis before infection of the CNS can be achieved (Fraser *et al.*, 1996). Suggested sites for extraneural agent replication include the follicular dendritic cells (FDC) found in lymphoid tissue and in the white pulp of the spleen (Fraser & Farquhar, 1987; Fraser *et al.*, 1992).

SCID mice have an autosomal recessive mutation that results in an immunodeficiency status which includes the production of non-functional FDC (Szakal *et al.*, 1990). SCID mice can therefore be used as an indicator of the involvement of the lymphoreticular system in the pathogenesis of scrapie. For example, it has been observed that SCID mice injected i.p. with 1% homogenates of ME7-infected mouse brain are resistant to infection because of the lack of agent replication in the spleen (H. Fraser, unpublished). In this present study, four SCID mice and four congenic immunocompetent CB20 mice were equally susceptible to infection following i.c. injection with the ME7 strain of scrapie agent, the mean incubation periods being 165 (4.0) and 172 (1.7) days respectively. Seventeen CB20 mice inoculated by scarification became infected, with a mean incubation period of 343 (2.8) days but 30 SCID mice did not. These mice were scarified at the medial surface of the right thigh, as already described. Each inoculum was 6 µl of a 1% homogenate of ME7-infected mouse brain which contains $\sim 10^{6.5}$ ID₅₀/g when titrated in immunocompetent mice by the scarification route (Table 1). The absence of disease in the SCID mice indicates that infectivity does not reach the CNS directly by either the bloodstream or peripheral nerves following inoculation by scarification but that it is probably transported by the lymphatic system to the lymph nodes and spleen within which infectivity replicates in immunocompetent animals before travelling to the CNS via the splanchnic nerves (Kimberlin & Walker, 1983).

Scarification must be regarded as a highly effective means of transmission, given that these experiments have shown its efficiency to be little different to that of the i.p., i.v. or p.v. routes. Indeed, it may be argued that scarification is probably more effective than the other routes because not all of the inoculum applied during the scarification procedure could possibly enter the host. It would appear therefore that the unhealed umbilical stump or skin lesions represent sites through which infectivity could efficiently gain access to sheep. The results also suggest that protection from skin trauma in the autopsy room or the laboratory, as is recommended customarily, is a sensible defence against acquiring CJD through occupational exposure.

References

- Brotherston, J. G., Renwick, C. G., Stamp, J. T. & Zlotnik, I. (1968). Spread of scrapie by contact to goats and sheep. *Journal of Comparative Pathology* **78**, 9–17.
- Brown, P., Preece, M. A. & Will, R. G. (1992). 'Friendly fire' in medicine: hormones, homografts, and Creutzfeldt–Jakob disease. *Lancet* **ii**, 24–27.
- Dickinson, A. G. & Fraser, H. (1979). An assessment of the genetics of scrapie in sheep and mice. In *Slow Transmissible Diseases of the Nervous System*, vol. 1, pp. 367–385. Edited by S. B. Prusiner & W. J. Hadlow. New York: Academic Press.
- Dickinson, A. G., Meikle, V. M. H. & Fraser, H. (1968). Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. *Journal of Comparative Pathology* **78**, 293–299.
- Dickinson, A. G., Stamp, J. T. & Renwick, C. C. (1974). Maternal and lateral transmission of scrapie in sheep. *Journal of Comparative Pathology* **84**, 19–25.
- Fraser, H. & Dickinson, A. G. (1968). The sequential development of the brain lesions of scrapie in three strains of mice. *Journal of Comparative Pathology* **78**, 301–311.
- Fraser, H. & Farquhar, C. F. (1987). Ionising radiation has no influence on scrapie incubation period in mice. *Veterinary Microbiology* **13**, 32–41.
- Fraser, H., Bruce, M. E., Davies, D., Farquhar, C. F. & McBride, P. A. (1992). The lymphoreticular system in the pathogenesis of scrapie. In *Prion Diseases of Humans and Animals*, pp. 308–317. Edited by J. Collinge, J. Powell & B. Anderton. Chichester: Ellis Horwood.
- Gajdusek, D. C. (1985). Subacute spongiform virus encephalopathies caused by unconventional viruses. In *Subviral Pathogens of Plants and Animals: Viroids and Prions*, pp. 483–544. Edited by K. Maramorosch & J. McKelvey. New York: Academic Press.
- Gordon, W. S. (1966a). Transmission of scrapie and evidence of spread of infection in sheep at pasture. *Report of a Scrapie Seminar Held at Washington DC from 27–30 January 1964*, pp. 8–18. Document ARS 91-53 of the Agricultural Research Service of the US Department of Agriculture.
- Gordon, W. S. (1966b). Review of work on scrapie at Compton, England, 1952–1964. *Report of a Scrapie Seminar Held at Washington DC from 27–30 January 1964*, pp. 19–40. Document ARS 91-53 of the Agricultural Research Service of the US Department of Agriculture.
- Greig, J. R. (1940). Scrapie: observations on the transmission of the disease by mediate contact. *Veterinary Journal* **96**, 203–206.
- Hadlow, W. J., Race, R. E., Kennedy, R. C. & Eklund, C. M. (1979). Natural infection of sheep with scrapie virus. In *Slow Transmissible Diseases of the Nervous System*, vol. 2, pp. 3–12. Edited by S. B. Prusiner & W. J. Hadlow. New York: Academic Press.
- Haralambiev, H., Ivanov, I., Vesselinova, A. & Mermerski, K. (1973). An attempt to induce scrapie in local sheep in Bulgaria. *Zentralblatt für Veterinärmedizin Reihe B* **20**, 701–709.
- Karber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologische Reihen versuche. *Archives of Experimental Pathology and Pharmacology* **162**, 480–483.
- Kimberlin, R. H. & Walker, C. A. (1983). Invasion of the CNS by scrapie agent and its spread to different parts of the brain. In *Virus non Connetionnels et Affections du Systeme Central Nerveux*, pp. 17–33. Edited by L. A. Court & F. Cathala. Paris: Masson.
- Marsh, R. F. & Hanson, R. P. (1979). On the origin of transmissible mink encephalopathy. In *Slow Transmissible Diseases of the Nervous System*, vol. 1, pp. 451–460. Edited by S. B. Prusiner & W. J. Hadlow. New York: Academic Press.
- Palsson, P. A. (1979). Rida (scrapie) in Iceland and its epidemiology. In

Slow Transmissible Diseases of the Nervous System, vol. 1, pp. 357–366. Edited by S. B. Prusiner & W. J. Hadlow. New York: Academic Press.

Pattison, I. H. & Millson, G. C. (1961). Experimental transmission of scrapie to goats and sheep. *Journal of Comparative Pathology* **71**, 171–176.

Pattison, I. H., Hoare, M. N., Jebbett, J. N. & Watson, W. A. (1974). Further observations on the production of scrapie in sheep by oral dosing with foetal membranes from scrapie-affected sheep. *British Veterinary Journal* **130**, ixv–ixvii.

Szakai, A. K., Kapasi, Z. F., Shultz, L. D., Burton, G. D., Kosco, M. H. & Tew, J. G. (1990). Reconstitution of follicular dendritic cell deficit in

SCID mice with isogenic and xenogenic bone marrow and lymphocytes. *Proceedings of the Tenth International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions*. Edited by B. A. Imhof.

Taylor, D. M. (1989). Bovine spongiform encephalopathy and human health. *Veterinary Record* **125**, 413–415.

Taylor, D. M. (1993). Inactivation of SE agents. *British Medical Bulletin* **49**, 810–821.

Received 11 January 1996; Accepted 14 March 1996